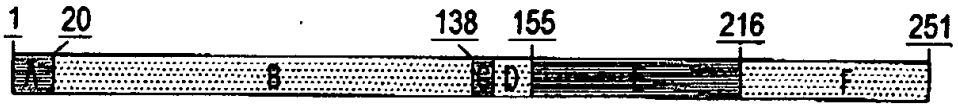


Fig. 1

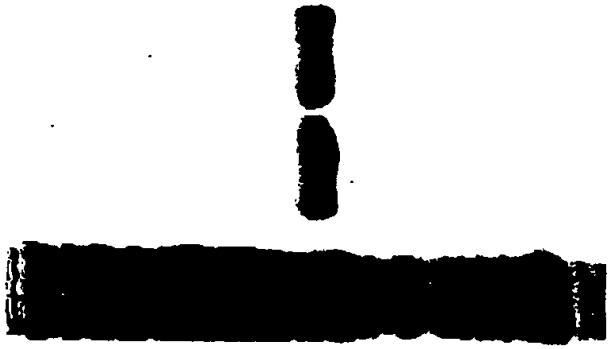


FIG - 2A

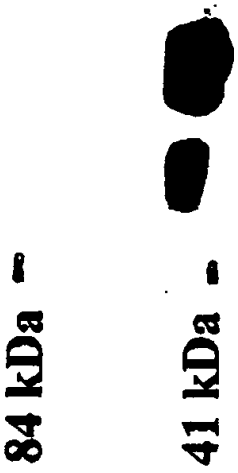
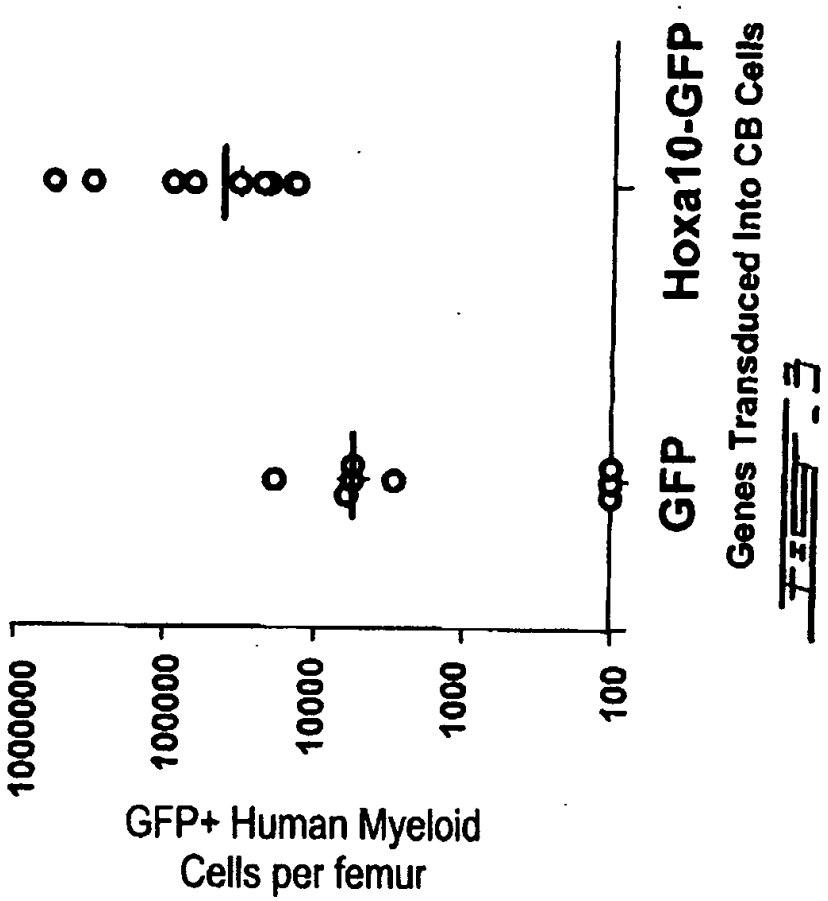


FIG - 2B



Genes Transduced Into CB Cells

FIG - 3

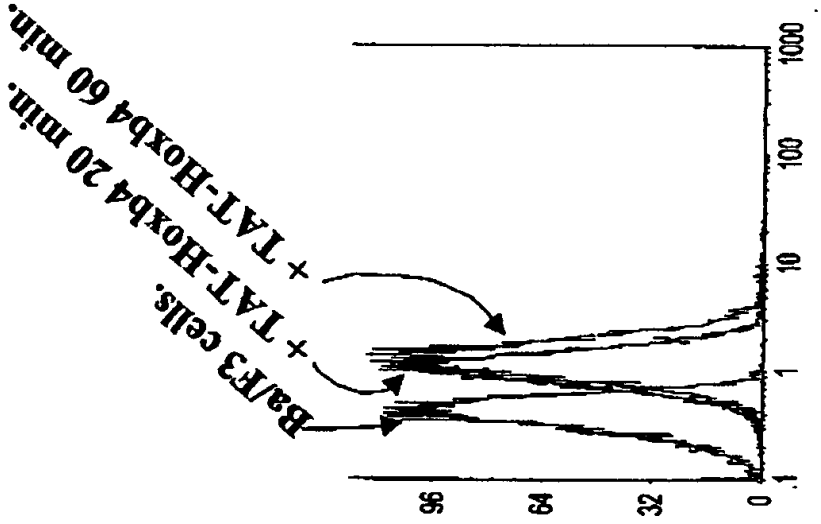


FIG - 2C

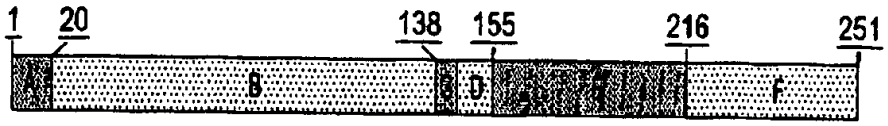


FIG. 4A

Mutant HOXB4 protein (letters refer to HOXB4 domains)	Proliferative effect on Rat-1 cells	Proliferative effect on d12-CFU-S (over controls)
A to F (wild-type)	++	↑200 X
C+D+E+F	++	No increase
A+B+C	No effect	No increase
A to F with point mutation in C (Trp>Gly)	No effect	No increase
A to F with point mutation in E (Asn>Ser)	No effect	No increase

FIG. 4B

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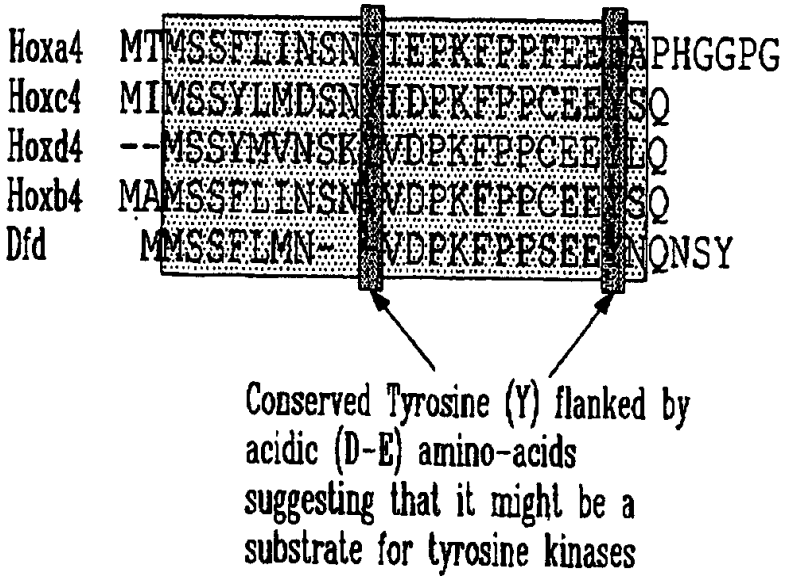
Domain A: Protein sequence


Hoxa4 MTMSSFLINSNIEPKFPPFEAPHGGPG
Hoxc4 MIMSSYLMDSNIDPKFPPCEESQ
Hoxd4 --MSSYMVNSKVDPKFPPCEELQ
Hoxb4 MAMSSFLINSNVDPKFPPCEESQ
Dfd MMSSFLMN-HVDPKFPPSEENQNSY
Conserved Tyrosine (Y) flanked by acidic (D-E) amino-acids suggesting that it might be a substrate for tyrosine kinases

Domain B: Not conserved but contains :

-a long proline stretch (n=15)
-4 tyrosines
-a potential site for phosphorylation by cAMPprotein kinase
-a potential site for phosphorylation by casein kinase II
-a potential site for phosphorylation by PKC

FIG 5

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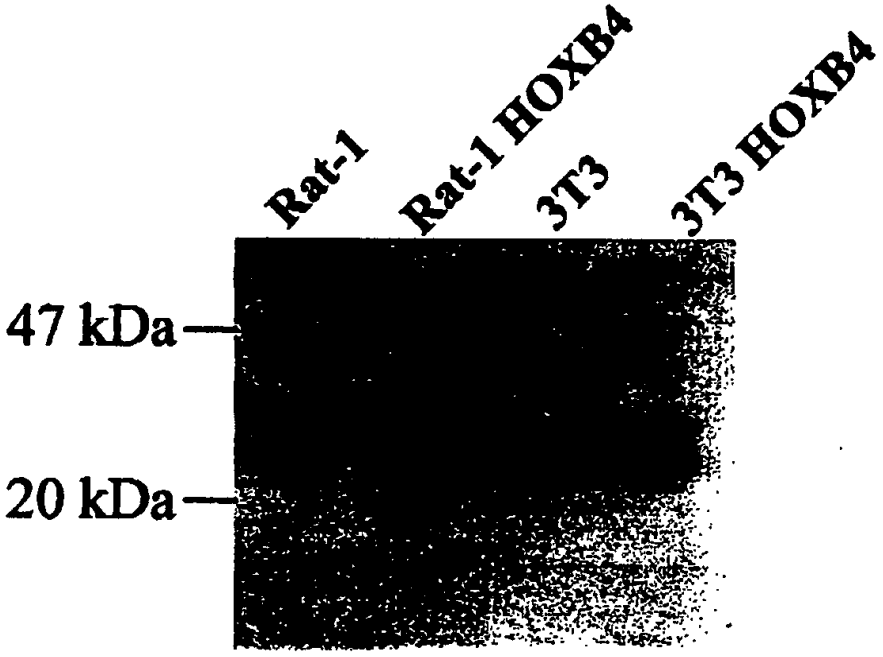

Fig. 6

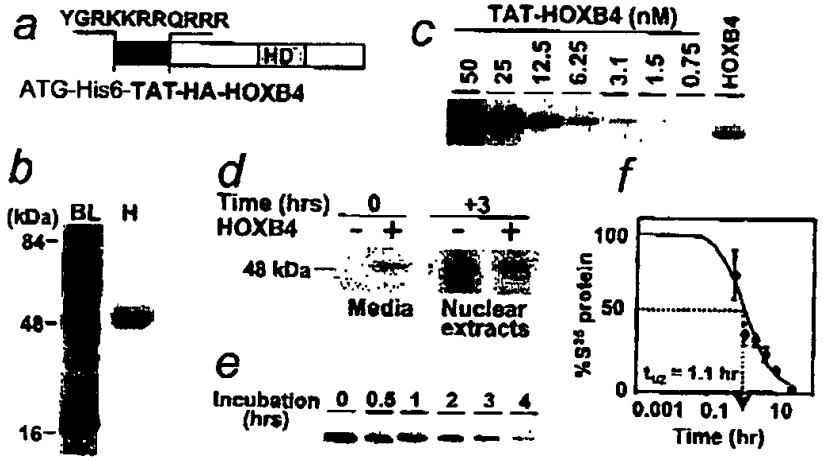
a
YGRKKRRQRRR
HD
ATG-His6-TAT-HA-HOXB4
b
(kDa) BL H
84-
48-
16-
c
TAT-HOXB4 (nM)
50 25 12.5 6.25 3.1 1.5 0.75 HOXB4
d
Time (hrs) 0 +3
HOXB4 - + - +
48 kDa
Media
Nuclear extracts
e
Incubation (hrs) 0 0.5 1 2 3 4
f
100
50
0
%S35 protein
t1/2 = 1.1 hr
0.001 0.1 10
Time (hr)

Fig. 7

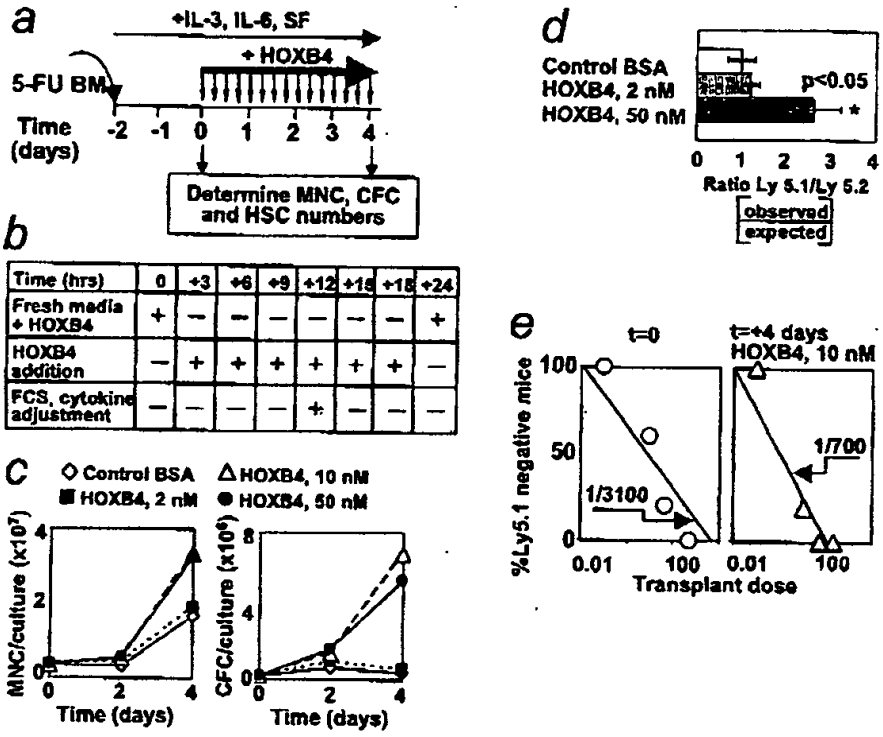
a
+IL-3, IL-6, SF
+ HOXB4
5-FU BM
Time (days)
-2 -1 0 1 2 3 4
Determine MNC, CFC and HSC numbers
b
Time (hrs) 0 +3 +6 +9 +12 +15 +18 +24
Fresh media + HOXB4 + – – – – – – +
HOXB4 addition – + + + + + + –
FCS, cytokine adjustment – – – – + – – –
c
Control BSA
HOXB4, 2 nM
HOXB4, 10 nM
HOXB4, 50 nM
MNC/culture (x10⁷)
Time (days)
CFC/culture (x10⁴)
Time (days)
d
Control BSA
HOXB4, 2 nM
HOXB4, 50 nM
p<0.05
Ratio Ly 5.1/Ly 5.2
observed
expected
e
t=0
t=+4 days
HOXB4, 10 nM
%Ly5.1 negative mice
1/3100
1/700
Transplant dose

Fig. 8

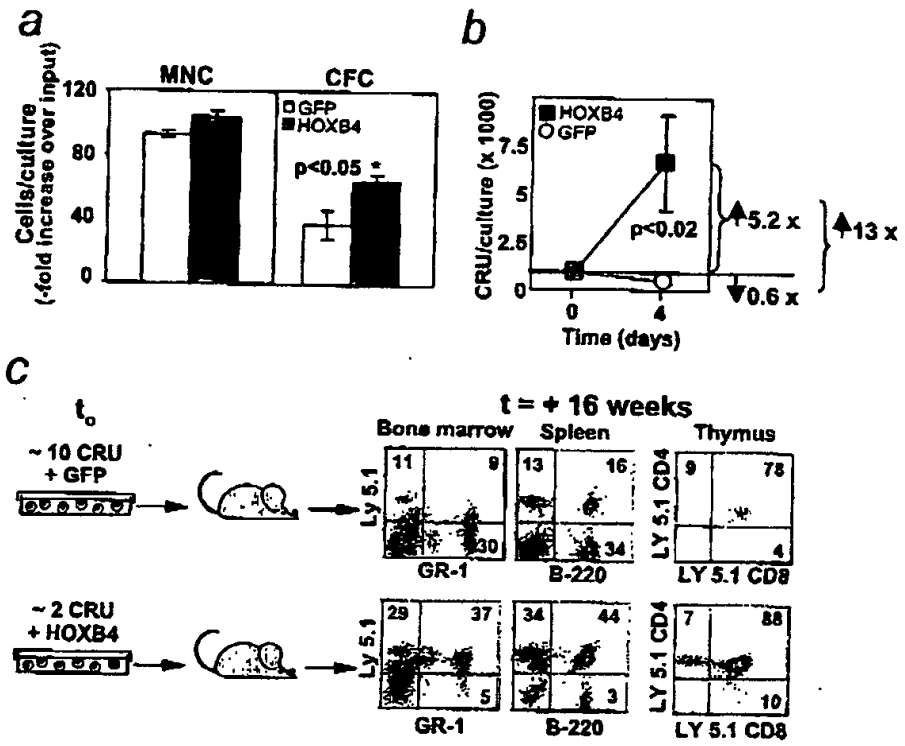
a
MNC
CFC
GFP
HOXB4
p<0.05 *
Cells/culture (-fold increase over input)
120
80
40
0
b
HOXB4
GFP
p<0.02
5.2 x
13 x
0.6 x
CRU/culture (x 1000)
7.5
5
2.5
0
0
4
Time (days)
c
t0
~ 10 CRU + GFP
~ 2 CRU + HOXB4
t = + 16 weeks
Bone marrow
Spleen
Thymus
Ly 5.1
GR-1
B-220
LY 5.1 CD4
LY 5.1 CD8
11
9
30
13
16
34
9
78
4
29
37
5
34
44
3
7
88
10

Fig. 9